SHORT COMMUNICATION

Experimental Vector Incompetence of a Soft Tick, *Ornithodoros sonrai* (Acari: Argasidae), for Crimean-Congo Hemorrhagic Fever Virus

LANCE A. DURDEN,[1] THOMAS M. LOGAN, MARK L. WILSON,[2] AND KENNETH J. LINTHICUM[3]

Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702

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ABSTRACT Adults and nymphs of a soft tick, *Ornithodoros sonrai* Sautet & Witkowski, were allowed to feed on suckling mice that had been experimentally infected with Crimean-Congo hemorrhagic fever (CCHF) virus (IbAr 10200 strain). The mean viral titer of mouse blood at the time of tick feeding was $10^{3.2}$ plaque-forming units (PFU) per ml. Samples of ticks were assayed on 12 occasions between days 0 and 31 after the viremic blood meal. Mean CCHF viral titers were $10^{2.1}$ PFU per tick immediately after the viremic meal but declined to $10^{1.2}$ PFU per tick after 2 d, and no virus was detected beyond 8 d. The percentage of ticks with detectable virus was 92% (22/24) immediately after the viremic meal, but then declined to 20% (2/10) after 4 d and to 0% (0/44) after 11 or more days. Ticks were allowed to feed on sets of three naive suckling mice on days 0, 2, 5, 8, 11, 14, 21, and 28 after the viremic blood meal, but CCHF viral transmission did not occur. Similarly, no transovarial transmission of virus from CCHF virus-exposed *O. sonrai* to their progeny was observed. These results strongly indicate that *O. sonrai* is not a vector of CCHF virus.

KEY WORDS *Ornithodoros sonrai*, Crimean-Congo hemorrhagic fever virus, vector competence

CRIMEAN-CONGO HEMORRHAGIC fever (CCHF) virus (genus *Nairovirus*, family Bunyaviridae) is an RNA arbovirus that can cause serious, potentially fatal, illness in humans (Watts et al. 1988). It is widely distributed across much of Africa and Eurasia, where it typically causes inapparent infections in many species of vertebrates that serve as reservoir hosts (Wilson et al. 1990). Human infections result from contact with infected vertebrate or tick body fluids, and by bites from infected ticks (Watts et al. 1988). Isolations of CCHF virus have been made from at least 27 species of hard ticks (Ixodidae), most of which parasitize two or three different hosts during their life cycles; some *Hyalomma* spp. ticks are efficient laboratory vectors (Hoogstraal 1979; Watts et al. 1988; Logan et al. 1989, 1990). CCHF virus also has been isolated from two species of multihost soft ticks (Argasidae): *Argas persicus* (Oken) in Uzbekistan and North Africa and *Ornithodoros lahorensis* Neumann in Iran (Hoogstraal 1979, 1980; Camicas et al. 1986; Watts et al. 1988). However, CCHF virus was detectable for just 1 d and failed to replicate in three taxa of soft ticks (*Argas walkerae* Kaiser & Hoogstraal, *Ornithodoros porcinus porcinus* Walton, and *Ornithodoros savignyi* [Audouin]) after they had received intracoelomic inoculations of this virus (Shepherd et al. 1989). If soft ticks are involved in CCHF virus transmission cycles, then this phenomenon presents a perplexing new dimension to the epidemiology of CCHF virus and disease (Hoogstraal 1979, 1980). The large number of hosts parasitized and the extended longevity of most species (including *Ornithodoros sonrai* Sautet & Witkowski) could influence control programs, the rate and mode of transmission, and the period of endemicity for a pathogenic arbovirus transmitted by soft ticks. Because there currently is insufficient knowledge concerning the potential for soft ticks to transmit CCHF virus, experiments were conducted to determine if *O. sonrai*, a species occurring in CCHF-endemic areas of North and West Africa (Colas-Belcour & Rageau 1962, Brès

[1] Current address: Institute of Arthropodology and Parasitology, Landrum Box 8056, Georgia Southern University, Statesboro, GA 30460.

[2] Yale Arbovirus Research Unit, Department of Epidemiology and Public Health, Yale University School of Medicine, 60 College St., New Haven, CT 06510.

[3] Current address: U.S. Army Medical Component, Armed Forces Research Institute of Medical Sciences, 315–16 Rajvithi Rd., Bangkok, Thailand.

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et al. 1967), was capable of transmitting this virus to suckling mice under laboratory conditions. In Senegal, *O. sonrai* is a known vector of Bandia virus, which, like CCHF virus, belongs to the genus *Nairovirus* (Brès et al. 1967, Taufflieb et al. 1968). Chikungunya virus (genus *Alphavirus*, family Togaviridae) also has been isolated from *O. sonrai* from Senegal, but laboratory experiments demonstrated that this tick was not an efficient vector of that pathogen (Camicas et al. 1978).

Materials and Methods

A laboratory colony of *O. sonrai* ticks was established from wild-caught specimens excavated from a mammal burrow in Bandia forest, Senegal. The colony was kept in Nalgene jars with screw-cap lids whose centers had been removed and replaced with fine cloth mesh. Sterilized sea sand was added to the bottom of each jar to a depth of ≈2 cm. Suckling mice (5–7 d old) were anesthetized with ketamine hydrochloride and placed on the sand at 1–10 wk intervals to provide a blood meal for maintaining the colony. Nymphal and adult ticks were removed from the colony for transmission experiments, which were undertaken in biocontainment suites (BL-3) at room temperature (≈21°C).

To initiate host infections, outbred Harlan-Sprague ICR strain suckling mouse littermates were inoculated intraperitoneally with $10^{3.5}$ plaque-forming units (PFU) of CCHF virus (IbAr 10200). This viral strain was isolated from the hard tick *Hyalomma excavatum* Koch in Nigeria and was used in our experiments after four suckling mouse brain passages (Logan et al. 1989). A total of 150 adult and nymphal ticks was allowed to engorge on six of these anesthetized mice 4–5 d after virus inoculation. Immediately after tick feedings, 0.1 ml of blood was collected by cardiac puncture from each mouse before it was euthanized with CO_2. Each blood sample was mixed 1:10 in diluent (10% fetal bovine serum in Medium 199 with Hanks' salts and antibiotics) and frozen at −70°C until assayed for virus. Plaque assays on confluent monolayers of 2- to 3-d-old SW-13 cells using serial 10-fold dilutions of samples were performed to establish blood viral titers (Logan et al. 1990).

On 12 occasions, between days 0 and 31 after the infectious blood meal, ticks were triturated individually in 1 ml of diluent, and the tick suspensions were frozen as described for blood samples. At a later date, tick suspensions were thawed, centrifuged at 1,000 × *g* for 10 min at 4°C, and assayed for virus as described above. The remaining ticks were allowed to feed on groups of three different naive suckling mice on days 1, 2, 5, 8, 11, 14, 21, and 28 after the viremic blood meal. These suckling mice were monitored for death or other signs of CCHF infection.

Table 1. Mean titer of CCHF virus in *Ornithodoros sonrai* soft ticks that fed on suckling mice with mean viremias of $10^{3.2}$ PFU per milliliter of blood

D after feed	No. of ticks tested	Ticks positive (%)	Mean CCHF viral titer ($\log_{10}$PFU) of positive ticks
0	24	92	2.1
1	12	83	1.5
2	12	67	1.2
4	10	20	1.0
6	8	0	—
8	6	17	1.0
11	6	0	—
14	6	0	—
18	6	0	—
22	8	0	—
26	8	0	—
31	10	0	—

Blood (1 ml) was collected by cardiac puncture from each surviving mouse after 21 d; mice were anesthetized and later euthanized with CO_2 during this process. Blood samples were allowed to clot at room temperature and sera were removed and stored at −20°C before being assayed for antibody to CCHF virus using both an enzyme-linked immunosorbent assay (ELISA) with CCHF anti-mouse horseradish peroxidase conjugates, and a plaque-reduction neutralization test (PRNT) (Earley et al. 1967).

Eggs were obtained from three female *O. sonrai* that had previously fed on viremic mice. The resulting larval ticks from these eggs were pooled (n = 192) and assayed for CCHF virus as previously described.

Results

The mean CCHF viral titer was $10^{3.2}$ PFU per ml of blood for virus-inoculated suckling mice immediately after tick feeding. Ticks sampled immediately after they had fed on these mice contained a mean of $10^{2.1}$ PFU per positive tick, but titers decreased six-fold after 24 h (Table 1). Viral titers in ticks continued to decrease rapidly and virus was not detected more than 8 d after the viremic blood meal. Similarly, the percentage of CCHF virus-positive ticks decreased from 92% (22/24) immediately after feeding to 20% (2/10) 4 d after feeding, and to 0% (0/44) after 11 or more days (Table 1). None of the 24 naive suckling mice that were fed on by CCHF virus-exposed ticks showed signs of CCHF infection, and none died. Similarly, antibody was not demonstrated by ELISA or PRNT in sera of mice that were fed on by virus-exposed ticks. All assay results for virus in the pooled progeny of female ticks that fed on viremic mice were also negative.

Discussion

Results of the laboratory transmission tests described here strongly indicate that the soft tick

O. sonrai is not involved in natural transmission cycles of CCHF virus. Although *O. sonrai* is capable of transmitting at least one arbovirus (Bandia virus) and inhabits CCHF-endemic regions of Africa, it failed to transmit CCHF virus to naive suckling mice. These results contrast with the laboratory transmissions of CCHF virus by hard ticks (Hoogstraal 1979, 1980; Camicas et al. 1986; Watts et al. 1988; Logan et al. 1989, 1990; Wilson et al. 1990; Okorie 1991; Shepherd et al. 1991), including the contemporaneous successful transmission of the same CCHF viral strain to naive suckling mice by *Hyalomma truncatum* Koch hard ticks in our laboratory. Although there is a lack of data on laboratory infection and transmission of CCHF virus by soft ticks, the results presented here, coupled with the limited number of CCHF virus isolations from soft ticks, indicate that their role in CCHF ecology may be limited (Shepherd et al. 1989). Similarly, we were unable to demonstrate vertical virus transmission from CCHF virus-exposed female *O. sonrai* to their progeny.

Although *O. sonrai* imbibed CCHF virus when fed on viremic suckling mice, the virus failed to replicate in the ticks. This indicates that CCHF viral isolates from soft ticks in nature (Hoogstraal 1979, 1980; Camicas et al. 1986; Watts et al. 1988) may be from ticks that had fed recently on viremic animals. Because CCHF virus failed to replicate in *O. sonrai* in our study, this tick may not be a biological vector of the virus.

The relatively high viral titers that were detected in ticks immediately after they had engorged on viremic mice, and the tendency of soft ticks to refeed frequently, further indicated that mechanical transmission of CCHF virus by these ticks might occur. However, we failed to record transmission even when ticks were allowed to refeed on naive mice a short time after their viremic blood meal. Perhaps some aspect of the physiology or biology of *O. sonrai* prevents this tick from transmitting CCHF virus mechanically.

Our failure to infect *O. sonrai* with CCHF virus indicates that this tick might possess a midgut virus barrier, or that a midgut CCHF virus receptor is absent. Another possibility is that in nature the strain (or strains) of CCHF virus that infects soft ticks may differ from strains infecting hard ticks and therefore also from the strain used in our transmission experiments.

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